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Chromosome Numbers of *Isodon* (Lamiaceae) in Japan

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Somatic chromosome numbers were counted in seven species and six varieties of *Isodon* occurring in Japan. All of the taxa had $2n = 24$ chromosomes, indicating that they are diploid. For all taxa except *I. umbrosus* var. *hakusanensis* and *I. japonicus,* chromosome numbers are reported here for the first time. The results suggest that diversification in Japanese taxa of *Isodon* has occurred at the diploid level, even though some reticulation had been suggested by a preliminary molecular study.

Key words: chromosome number, *Isodon*, Japan, Lamiaceae

Isodon (Benth.) Schrad. ex Spach (Lamiaceae) comprises approximately 100 species having a calyx with one large upper tooth and four small lower ones, as well as those having a calyx with five equal or subequal teeth, or three upper teeth and two lower ones (Li 1988). The genus is widely distributed from the Far East to Africa; in Japan, seven species and six varieties are currently known (Murata & Yamazaki 1993). Corolla sizes of the Japanese taxa of *Isodon* vary greatly and are known to correspond well to their pollinator species (Suzuki 1992a, b, Suzuki & Akazome 2000), offering good examples of plant-pollinator mutualism.

At present, only limited amount of information on chromosome numbers are available for *Isodon* (Darlington & Wylie 1955, the Index to Plant Chromosome Numbers 1956 – 2000). To date, chromosome counts are published for only two taxa of the genus in Japan; Suzuka (1950) reported the chromosome number of *Plectranthus japonicus* (Burm. f.) Koidz. [= *Isodon japonicus* (Burm. f.) Hara] to be $2n = 24$, and Wakabayashi (1973) reported $2n = 24$ for *Rabdosia umbrosa* (Maxim.) Hara var. *hakusanensis* (Kudo) Hara [= *Isodon umbrosus* (Maxim.) Hara var. *hakusanensis* (Kudo) K. Asano].

From a preliminary phylogenetic study using sequence variations of chloroplast DNA, origins of some Japanese taxa of *Isodon* are revealed to be complex, probably involving reticulate evolution (Dohzono *et al.* 2004). For example, *I. inflexus* has two different haplotypes of cp DNA, which belong to different clades, suggesting that the species originated from different two lineages. Because, in plants, reticulation is very often accompanied by polyploidization (Hegarty & Hiscock 2005), it is possible that allopolyploidization is relevant to the origin of some Japanese taxa of *Isodon*. Accordingly, chromosome numbers are essential information for uncovering the speciation process in the Japanese taxa of *Isodon*. In this study, we report the somatic chromosome numbers of *Isodon* occurring in Japan.

Materials and Methods

Living stocks were collected from natural populations of seven species and six varieties of *Isodon* in Japan (Table 1), and were cultivated in pots filled with soil in the nursery of the University of Tokushima. Taxonomic treatments of the taxa follow Murata & Yamazaki (1993).

For the observation of somatic chromosomes, root tips were pretreated in a 2 mM 8-hydroxyquinoline aqueous solution for 3.5 hours at room temperature. The root tips were then fixed with a mixture of 100 % ethanol and acetic acid (3:1) for 30 minutes and preserved at 5 °C until observation. For observation, root tips were macerated with a mixture of 1N HCl and 45 % acetic acid (2:1) at 60 °C for 30 seconds, then stained in 2 % aceto-orcein solution for an hour. The meristematic parts of the root tips were squashed in 2 % aceto-orcein on slide glass and were observed by using a microscope at 1500 magnification. Chromosome numbers of at least three cells for each individual were counted at somatic metaphase.

Results and Discussion

All 13 taxa investigated in this study had a chromosome number of $2n = 24$, representing diploid of $x = 12$ (Figs. 1, 2). All chromosomes of 13 taxa observed in this study were similar to each other in size. The lengths of the chromosomes varied from 0.7 to 1.4 μm. The centromeric positions of the chromosomes were obscure.

The chromosome counts in the present study are consistent with those of two previous studies reporting $2n = 24$ for *Isodon umbrosus* var. *hakusaensis* (Wakabayashi 1973) and *I. japonicus* (Suzuka 1950). For all taxa except *I. umbrosus* var. *hakusaensis* and *I. japonicus*, chromosome numbers are reported here for the first time. Two taxa closely related to the Japanese members, *Rabdosia excisa* (Maxim.) Hara [= *Isodon excisus* (Maxim.) Kudo] and *R. japonica* var. *glaucocalyx* Maxim. [= *Isodon japonicus* var. *glaucocalyx* (Maxim.) H. W. Li], also have chromosome number of $2n = 24$ (Sokolovskaya *et al.* 1986). According to a previous series of the Index to Plant Chromosome Numbers

TABLE 1. List of taxa, localities, and chromosome numbers of *Isodon* examined in this study.

Taxon	Locality	Chromosome number ($2n$)
I. effusus	Mt. Ogasa-yama, Fukuroi City, Shizuoka Pref.	24
I. inflexus	Mt. Bizan, Tokushima City, Tokushima Pref.	24
	Tsushima Islands, Nagasaki Pref.	24
I. japonicus	Ohgisawa, Ohmachi City, Nagano Pref.	24
	Toyama, Naka Town, Tokushima Pref.	24
I. longitubus	Entrance of Mt. Shakaga-dake, Yabe Town, Fukuoka Pref.	24
	Sawadani, Naka Town, Tokushima Pref.	24
I. shikokianus	Mts. Ishiduchi-san, Saijyo City, Ehime Pref.	24
var. *intermedius*	Mt. Kotsu-zan, Yoshinogawa City, Tokushima Pref.	
var. *occidentalis*	Mt. Tadagatake, Obama City, Fukui Pref.	24
I. tricocarpus	Mts. Izumigatake, Sendai City, Miyagi Pref.	24
I. umbrosus	Abe Pass, Shizuoka City, Shizuoka Pref.	24
var. *excisinflexus*	Atsumi Spa, Atsumi Town, Yamagata Pref.	24
var. *hakusanensis*	Ohgisawa, Ohmachi City, Nagano Pref.	24
var. *latifolius*	Yashajin Pass, Minami Alps City, Yamanashi Pref.	24
var. *leucanthus*	Ichinose Heights, Enzan City, Yamanashi Pref.	24

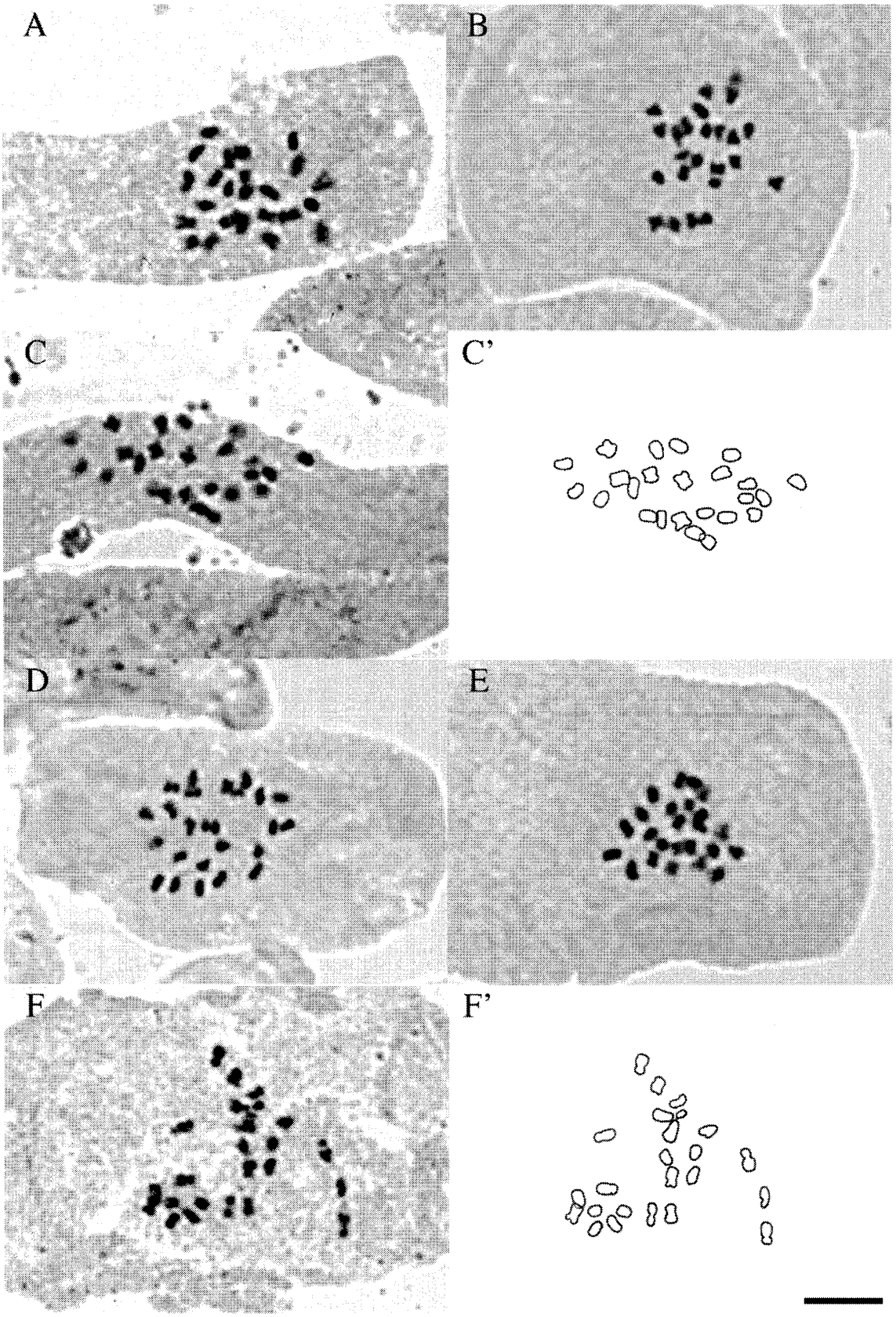

FIG. 1. Microphotographs of somatic chromosomes at metaphase of Japanese *Isodon*. A: *Isodon effuses*. B: *I. Inflexus*. C: *I. Japonicus*. D: *I. longitubus*. E: *I. shikokianus* var. *shikokianus*. F: *I. shikokianus* var. *intermedius*. C', F': explanatory drawings of C and F, respectively. Scale bar represents 5 μm.

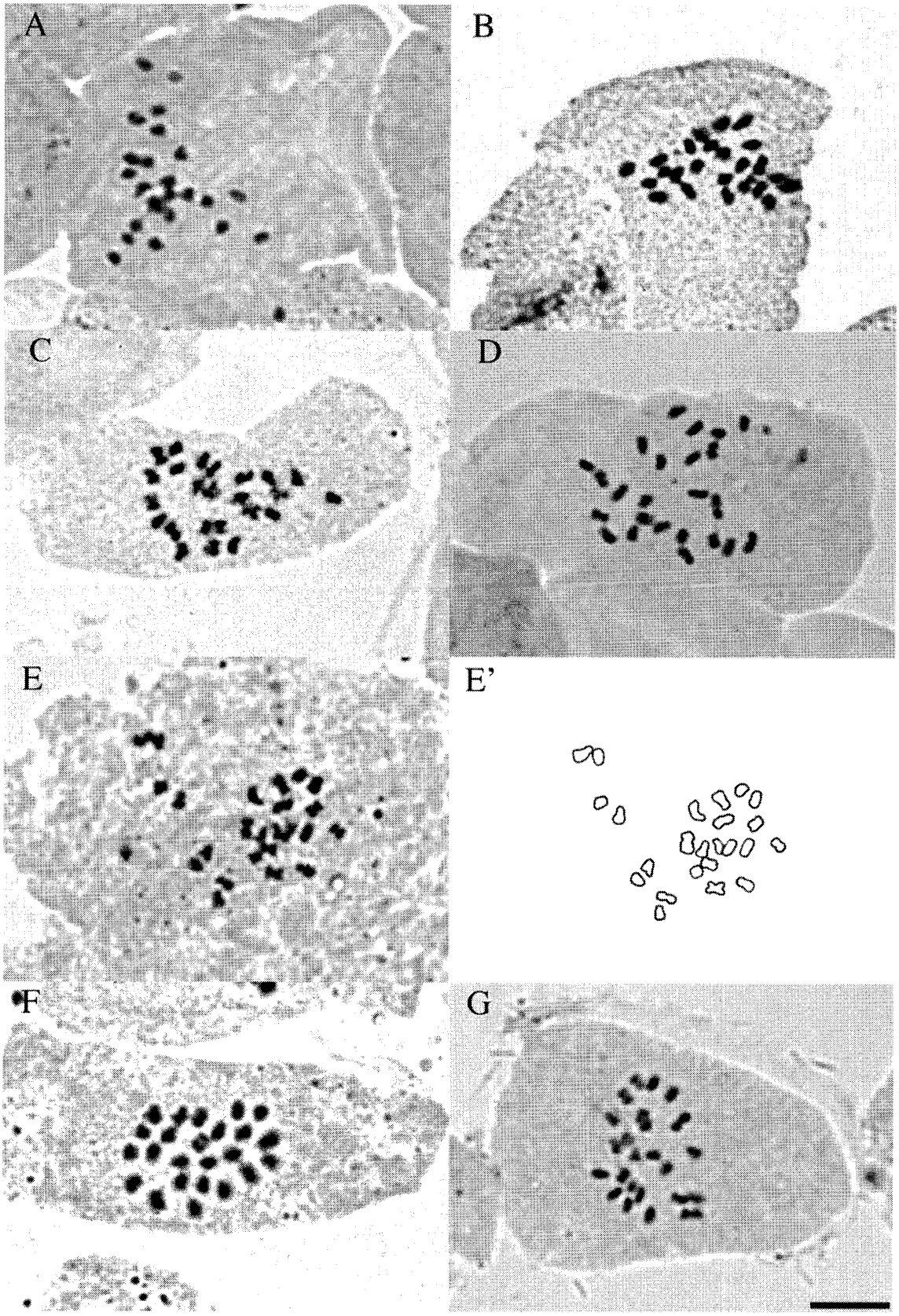

FIG. 2. Microphotographs of somatic chromosomes at metaphase of Japanese *Isodon*. A: *I. shikokianus* var. *occidentalis*. B: *I. tricocarpus*. C: *Isodon umbrosus* var. *umbrosus*. D: *I. umbrosus* var. *excisinflexus*. E: *I. umbrosus* var. *hakusanensis*. F: *I. umbrosus* var. *latifolius*. G: *I. umbrosus* var. *leucanthus*. Scale bar represents 5 μm. E': explanatory drawing of E. Scale bar represents 5 μm.

(1956 - 2000) and Darlington & Wylie (1955), only $2n = 24$ (or $n = 12$), 28 [only in *Plectranthus calycinus* Benth. (= *Isodon calycinus* (Benth.) H. W. Li) (de Wet 1958)] and 42 [only in *I. ramosissimus* (Hook. f.) Codd (Morton 1998)] are reported for *Isodon sensu* Li (1988), suggesting that all Japanese taxa of *Isodon* are diploid. This is supported by the results of enzyme electrophoresis, by which additional gene duplications were not detected (Maki *et al.* unpublished data). A recent molecular phylogenetic study on Ocimeae revealed that *P. calycinus*, with a chromosome number of $2n = 28$, is not contained in the clade comprised of *Isodon* (Paton *et al.* 2004), although *I. ramosissinus* with $2n = 42$ has not been examined, and its phylogenetic position remains still unclear. It is probable that species having a basic chromosome number of $x = 14$ are phylogenetically independent of *Isodon.* If $x = 14$ is another basic chromosome number of *Isodon*, *I. calycinus* is diploid and *I. ramosissinus* is triploid.

Consequently, it is unlikely that polyploidy plays a role in the speciation of the Japanese taxa of *Isodon,* and it is probable that their diversification occurs at the diploid level. In other words, the putative reticulative evolutions suggested in the group would have occurred at the diploid level. We should consider the possibility of introgressive hybridization or diploid hybrid speciation in the taxa suspected of reticulative evolution in the preliminary study. Because several hybrids between the Japanese taxa of *Isodon* have been reported (Murata & Yamazaki 1993), natural hybridization may have played a major role in the evolution of the taxa. It is important to assess to what extent these hybrids have fertility.

For investigation of natural hybridization or hybrid speciation in plants, molecular works using more than one kind of marker are very effective (Hegarty & Hiscock 2005). Molecular phylogenetic studies on *Isodon* in Japan employing sequence variations of chloroplast and nuclear DNA are now in progress, and will elucidate the speciation process in the taxa.

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